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Biomaterial-assisted cell therapy in osteoarthritis: From mesenchymal stem cells to cell encapsulation

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A B S T R A C T

Osteoarthritis (OA) is a degenerative and inflammatory joint disease that affects the cartilage, subchondral bone, and joint tissues. Although current drug therapies can provide a degree of symptomatic relief from pain, they fail to prevent joint damage. Mesenchymal stem/stromal cells (MSCs) have generated significant interest in terms of medical applications because they exert their therapeutic properties by secretion of bioactive factors that have potent immunomodulatory, antiapoptotic, antifibrotic, and anti-inflammatory effects. However, intra-articular injection of MSCs has major limitations including cell death upon injection and massive leakage from the injection site. Encapsulation of MSCs has therefore been developed as a way to overcome these limitations and to deliver therapeutic bioactive factors in several pathologies. In this review, we first briefly highlight the main therapeutic properties of MSCs and their applications in OA treatment. We

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then focus on MSC encapsulation and the current advances this strategy offers for the treatment of OA.

Introduction

Osteoarthritis (OA) is the most common joint disease in humans and one of the leading causes of disability in adults. According to the 2016 Global Burden of Disease (GBD) study, the world prevalence of OA in adults is estimated to amount to more than 300 million cases, and this number is likely to continue to increase as the population ages [1]. The risk of OA depends on age, gender, heredity factors, repetitive joint stress, and metabolic disorders [2,3]. Young or middle-aged patients can also suffer from OA following joint injuries and articular cartilage lesions when these are left untreated [4].

OA affects all joints, and it induces its main disabling symptoms when it affects load-bearing joints such as the hip and knees. OA is characterized by a progressive destruction of articular cartilage associated with subchondral bone sclerosis, osteophyte formation, and synovitis [5–7]. This inflammation leads to the production of catabolic and proinflammatory mediators such as cytokines, nitric oxide (NO), or prostaglandin (PG) E_2. This chronic and low-grade inflammation is responsible for the characteristic symptoms of OA such as pain and joint stiffness as well as the progression of the OA [8].

Current treatments primarily comprise oral administration of conventional painkillers including nonsteroidal anti-inflammatory drugs and opioid analgesics in particular; intra-articular (IA) injection of corticosteroids; and surgical treatments such as osteotomy, arthroplasty, and arthrodesis [3,9,10]. However, these treatments are only symptomatic, and they do not treat the underlying causes of OA nor do they prevent joint damage. In light of this, transformative therapeutic avenues need to be considered for prevention of the progression of OA, such as ways to address the inflammation-associated alteration of diseased articular tissues. Among the conceivable strategies to achieve this, the use of mesenchymal stem/stromal cells (MSCs) appears to be a promising option owing to their wide variety of biological properties that include anti-inflammatory, immunomodulatory, and anti-fibrotic effects [11].

For many years, these therapeutic activities of MSCs have been attributed to the secretion of various autocrine/paracrine factors including growth factors, cytokines, and chemokines. More recently, microvesicles and exosomes have also been shown to play important roles. These extracellular vesicles (EVs) act as carriers of the trophic factors, which can readily be released through their membrane [12]. Thus, they have been reported to be pivotal therapeutic agents responsible for the regenerative and immunomodulatory effects of MSCs as a result of their function in intercellular communication [13].

In keeping with their biological properties, several clinical trials have underscored the therapeutic potential of MSCs in OA treatment [5,14]. Despite these encouraging results, unfortunately, the injection of MSCs into the joint space has several limitations such as cell leakage and massive cell death upon injection [15–17]. To tackle these limitations and to improve the efficacy of MSCs in OA, their entrapment in particles before their injection has recently gained interest [16–18]. After providing a brief overview of the current knowledge regarding OA pathogenesis and the biological properties of MSCs, this review focuses on the recent advances related to hydrogel-based cell encapsulation and their potential application in OA.

OA pathogenesis

It is widely acknowledged that OA is not only a degenerative disease of cartilage but also a global joint disease with a significant inflammatory component. Indeed, OA is generally considered to be a chronic low-grade inflammatory disease with inflammation appearing in its early stages [19]. Synovitis is thus detectable at a very early stage even before the appearance of cartilage lesions. This inflammation is correlated with the early symptoms of OA such as pain, joint swelling, and disability [20]. It is accompanied by the infiltration of inflammatory cells, consisting primarily of macrophages as well as a smaller, yet quantifiable, number of T and B cells, mast cells, and NK cells [21]. The overexpression of

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proinflammatory mediators such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and NO in the synovium is observed, and this in turn affects the chondrocyte phenotype and behavior [22].

The innate immune system also has a major role in the pathogenesis of OA. Damage-associated molecular patterns (DAMPs), generated because of microtrauma or normal aging, activate the innate immune system by interacting with pattern-recognition receptors (PRRs) such as Toll-like receptors (TLR), and the receptor for advanced glycation end products (RAGE) present on the surface of immune cells. DAMPs are endogenous molecules that include products of extracellular matrix damage, alarmins, free fatty acids, and debris from dead cells. Numerous members of the TLR family (e.g., TLR1-7 and TLR9) have been detected in the synovium of patients with OA [23]. Among these, several studies have highlighted the importance of TLR2 and TLR4 in mediating catabolic responses [24–26]. Their activation by DAMPs leads to the production of proinflammatory mediators that induce OA progression by amplification of the synovitis and the degeneration of cartilage.

Because of its activation by DAMPs, the complement system is another entity implicated in the inflammation in the early stages of OA. The proteins C3a and C5b-9 are overexpressed in the synovial fluid and membrane of patients with OA, and these proteins induce catabolic and inflammatory responses of chondrocytes. Complement activation results in the formation of membrane attack complexes on chondrocytes, which either induces chondrocyte apoptosis or causes them to produce matrix-degrading enzymes, inflammatory mediators, and further complement effectors that promote joint damage [27].

Although their involvement has long been underestimated, macrophages are now increasingly being recognized as central players in inflammation-associated joint disorders. These cells can secrete either pro- or anti-inflammatory mediators depending on their M1 or M2 phenotype. The low-grade inflammation in OA in the presence of IFN-γ and TNF-α has hence been demonstrated to participate in macrophage polarization toward an M1 phenotype, which in turn produces TNF-α and IL-1β and take part in further inflammation and cartilage destruction [28].

Recent improvement in the understanding OA pathogenesis and notably the recently identified role of low-grade inflammation in the onset and progression of OA has led to considering new therapeutic strategies that are based on targeting of the early inflammatory processes, before cartilage degeneration takes place.

MSCs

Properties of MSCs

MSCs are present in many tissues in adults. They have been identified for the first time in the bone marrow [29], although they can also be isolated from several other tissues including the adipose tissue, synovium, muscles, tendons, and the vascular system [11]. The most frequently used MSCs in preclinical studies and clinical trials are those isolated from the bone marrow and adipose tissue [30]. MSCs are considered to be multipotent cells, as they are able to proliferate and differentiate into various cell lineages including chondrocytes, osteocytes, and adipocytes [11]. This capacity of MSCs to undergo chondrogenic differentiation first led to the use of MSCs in cartilage tissue engineering. MSCs were generally injected either directly into cartilage lesions or following differentiation into chondrocytes, with variable success [31–33]. Although improvements were often seen in terms of pain relief and function, the newly formed cartilage from MSCs did not meet the criteria for quality and durability of articular cartilage. The two major limitations in the use of MSCs for hyaline cartilage repair are (i) their limited ability to differentiate into cells with a stable chondrogenic phenotype that ultimately leads to the production of a fibrocartilaginous tissue instead of hyaline cartilage [34] and (ii) their propensity for hypertrophic chondrogenic differentiation leading to the formation of a type-X collagen-rich mineralized cartilage as observed during the endochondral ossification process [35]. The observed benefits of MSC injection in cartilage disorders are likely to be due to their paracrine effects that result from the secretion of growth factors, chemokines, or cytokines, rather than to their effects on chondrogenic differentiation. Indeed, by secreting a wide range of biological factors, MSCs may exert various functions such as antifibrotic or antiapoptotic effects in part by the production of hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF) [36]. In addition to these antiapoptotic and

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antifibrotic properties, their most widely recognized properties are their antiinflammatory and immunosuppressive functions that have been exploited in a large variety of disorders [36].

In the context of OA, where proinflammatory cytokines exert a central role, the use of MSCs is particularly promising. Indeed, in the presence of inflammatory mediators such as IFN-γ, TNF-α, IL-1β, or IL-1α, MSCs exert immunosuppressive properties [37].

The activation of MSCs induces the secretion of several mediators such as indoleamine 2,3-dioxygenase (IDO), NO, IL-6, or PGE_2, all of which affect the proliferation and the function of cells of both the innate and the adaptive immune systems [38].

This paracrine activity also promotes the functional switch of macrophages from a pro-to anti-inflammatory phenotype. Through different and yet to be fully defined molecular mechanisms, macrophage polarization is switched from M1 to M2, thereby inducing an increase in IL-10 and a decrease in TNF-α and proinflammatory cytokine secretion [39]. Among the various mechanisms that have been proposed, PGE_2 could play a role, as suggested by Maggini et al. Thus, MSCs may be able to secrete PGE_2 at sufficient levels to inhibit the production of TNF-α and IL-6 by M1 macrophages [40]. They also showed that in addition to preventing the production of proinflammatory cytokines by macrophages, they stimulate their apoptotic cell phagocytosis function.

The anti-inflammatory and immunosuppressive activities of MSCs are mediated by a large number of soluble factors. In recent years, however, among the large secretome of MSCs, there is a growing interest in the role of EVs. EVs, which include microvesicles and exosomes, provide communication between cells by contributing to the transfer of lipids, nucleic acids, and proteins. These EVs may exert effects similar to those of their parental MSCs. EVs could behave as extracellular stores that are able to distribute and gradually release anti-inflammatory factors for the treatment of OA [12]. Toh et al. have proposed a mechanism of action of EVs that involves restoration of homeostasis in bioenergetics, cell numbers, and immunomodulation [13].

All these properties make MSCs particularly promising candidates for OA treatment. Currently, autologous MSCs are used preferentially, as they limit the risk of inducing a potential immune response. However, it is well known that the capacity for differentiation and proliferation and the activity of MSCs decrease with age. Thus, allogeneic MSCs from young donors may be a more suitable source for the treatment of OA. Moreover, allogeneic MSCs have the advantage of being less expensive to obtain while they also allow for a higher level of homogeneity to be achieved [41].

Clinical trials

Proof-of-concept obtained from preclinical studies has led to several clinical trials being undertaken. However, several issues can stymie successful clinical translation. These issues pertain to the following: (i) the production of MSCs (e.g., difficulties in ensuring a pure culture of cells under xeno-free conditions), (ii) storage (e.g., problems with the cryopreservation of MSCs, as storage for more than 6 months leads to decreased cell proliferation and genetic instability), and (iii) the transportation of MSCs to the site of administration [42,43].

At the beginning of 2018, the clinicaltrials.gov site listed 44 open clinical trials (27 that had been completed and 17 that were in progress) addressing OA with IA injection of MSCs (Table 1). Almost all the trials were regarding OA of the knee, and there were only two for OA of the hip and one for OA of the ankle.

Different sources of MSCs are used, namely the bone marrow (19), adipose tissue (14), or umbilical cord (8). Bone marrow–derived MSCs (BM-MSCs) are the most studied MSCs owing to the ease of harvesting them. However, their potential for hypertrophic chondrogenesis and the decrease in cell numbers with age represent significant disadvantages. Adipose tissue–derived MSCs (ASCs) are a more abundant cell source that can also be harvested relatively easily with low donor site morbidity, reduced pain, and the presence of a high number of MSCs in lipoaspirate samples. Umbilical cord MSCs (UC-MSCs) represent a major source of allogeneic cells, as they can be harvested in a painless manner and unlimited numbers. BM-MSCs and ASCs have been demonstrated to have the same *in vitro* properties [44].

All these studies support the notion that IA injections of MSCs have ample merit for the treatment of OA. Evaluations based on the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) or Knee Injury and Osteoarthritis Outcome Score (KOOS) have generally indicated a

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Table 1
The main clinical trials in progress or completed using MSCs for OA treatment (ClinicalTrials.gov – February 2018).

Source of Stem Cells	Site	Autologous/Allogeneic	Trial phase	Nb of patients enrolled	Identifier	Status	Country
ASC	Knee	Autologous	I/II	18	NCT01809769	C	China
ASC	Knee	Autologous	I	4	NCT02544802	C	Taiwan
ASC	Knee	Allogeneic	I	18	NCT02641860	R	China
ASC	Knee	Allogeneic	I	10	NCT02966951	R	Jordan
ASC	Knee	Autologous	II	53	NCT02162693	C	China
ASC	Knee	Autologous	NP	26	NCT03000712	R	Korea
ASC	Knee	Autologous	I	18	NCT01585857	C	France
ASC	Knee	Autologous	II	153	NCT02838069	R	France
ASC	Knee	Autologous	NP	99	NCT03014401	R	USA
ASC	Knee	Autologous	I/II	30	NCT02142842	C	Vietnam
ASC	Knee	Allogeneic	I/II	56	NCT02784964	R	Taiwan
ASC	Knee	Autologous	II	28	NCT02674399	A	USA
ASC	Knee	Autologous	II	24	NCT02658344	C	Korea
ASC	Knee	Autologous	II	28	NCT02855073	R	China
BM-MSC	Knee	Autologous	II	40	NCT01504464	C	Iran
BM-MSC	Hip	Autologous	I	6	NCT01499056	C	Iran
BM-MSC	Knee	Autologous	I	6	NCT01207661	C	Iran
BM-MSC	Knee	Allogeneic	I/II	30	NCT01586312	C	Spain
BM-MSC	Knee	Autologous	I/II	12	NCT01183728	C	Spain
BM-MSC	Knee	Autologous	I/II	30	NCT02123368	C	Spain
BM-MSC	Knee	Allogeneic	II	60	NCT01453738	C	India
BM-MSC	Knee	Autologous	I/II	38	NCT02365142	A	Spain
BM-MSC	Knee	Allogeneic	II	72	NCT01448434	C	Malaysia
BM-MSC	Knee	Autologous	I/II	10	NCT01895413	C	Brazil
BM-MSC	Ankle	Autologous	I	6	NCT01436058	C	Iran
BM-MSC	Knee	Autologous	II	30	NCT02958267	C	USA
BM-MSC	Knee	Autologous	I	6	NCT00850187	C	Iran
BM-MSC	Knee	Autologous	II/III	60	NCT01873625	C	Iran
BM-MSC	Knee	Autologous	II	13	NCT02118519	C	Jordan
BM-MSC	Knee	Autologous	NP	30	NCT03014037	R	USA
BM-MSC	Knee	Autologous	I/II	12	NCT02351011	A	Canada
BM-MSC	Knee	Autologous	I/II	15	NCT01227694	C	Spain
BM-MSC	Knee	Autologous	I	61	NCT01485198	C	Mexico
BM-MSC	Hip/Knee	Autologous	NP	12	NCT01601951	C	USA
BM-MSC	Knee	Autologous	NP	20	NCT03130335	R	USA
Placenta	Knee	Allogeneic	II	1	NCT03028428	A	China
UC-MSC	Knee	Allogeneic	I/II	60	NCT03166865	R	China
UC-MSC	Knee	Allogeneic	I	10	NCT02963727	R	Jordan
UC-MSC	Knee	Allogeneic	I	20	NCT02291926	C	China
UC-MSC	Knee	Allogeneic	I/II	30	NCT02580695	A	Chile
UC-MSC	Knee	Allogeneic	NP	60	NCT03337243	R	USA
UC-MSC	Knee	Allogeneic	III	104	NCT01041001	C	Korea
UC-MSC	Knee	Allogeneic	III	103	NCT01626677	C	Korea
UC-MSC	Knee	Allogeneic	I/II	12	NCT01733186	C	USA

ASC: adipose tissue–derived stem cells; BM: bone marrow; UC: umbilical cord; R: recruiting; C: completed study; A: active; Nb: number; NP: Not provided.

reduction in pain and an improvement in joint function. A significant improvement in knee cartilage thickness was also observed by magnetic resonance imaging (MRI) [45].

These clinical trials also demonstrated the safety of IA injection of MSCs, with only a few cases of pain in the first few hours following their injection [45]. No long-term side effects have been reported.

Injected MSCs are mostly autologous, although an increasing number of studies are using allogeneic cells (around one-third). Many of the clinical trials using allogeneic MSCs have revealed an excellent level of tolerance to these cells. Side effects, which were always minor, occurred at the same rate for both allogeneic and autologous MSCs [41]. A randomized controlled trial assessed the feasibility and safety of treating OA with allogeneic MSCs [46]. These cells, being more logistically convenient than autologous MSCs, probably warrant further clinical analyses.

As an example of published clinical trial, Orozco et al. treated 12 patients with OA whose knees were severely affected (Kellgren and Lawrence grades II to IV). Autologous BM-MSCs (40 million) were injected after a 3-week *in vitro* proliferation step [47]. After 1 year, the patients exhibited an improvement and a gradual decline (65–78%) in functional discomfort and pain. In addition, MRI T2

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mapping revealed a significant decrease (27%) in the Poor Cartilage Index (PCI), with improvement in the cartilage quality in 11 of the 12 volunteers. As a second example, in 2016, the first phase of the ADIPOA trial was completed [48]. A single injection of autologous ASCs was administered to 18 patients with severe primary knee OA. Three doses of ASCs (2×10^6, 10×10^6, and 50×10^6) were administered as IA injections. The initial results revealed a positive response in 80% of the subjects, with an increased level of functionality and pain relief 9 months after the injection. The most substantial improvements were noted in patients who received 2×10^6 ASCs in total. Thus, the authors concluded that the therapeutic effect did not correlate with the number of the injected MSCs. The phase II for this trial, which assesses the effects of MSCs on the progression of the disease at the molecular and histological level, is ongoing with 150 patients.

These clinical trials have revealed promising outcomes, with MSC injections leading to a decrease in the level of pain and disability, as well as minimal side effects for patients.

Even if the quality of clinical trials is improving, some of them suffer from risk of bias related to a lack of randomization and blinding, small number of cases, or a short follow-up period. Among the 44 trials listed in Table 1, 25 can be classified as level I evidence according to the Journal of Bone and Joint Surgery (JBJS) level-of-evidence rating scale [49], corresponding to randomized controlled trials. Most of the time, the control group is treated with an IA injection of hyaluronic acid. Seventeen trials are also set up under double or more blinding conditions, thus limiting bias in the evaluation of placebo effect. However, the low number of patients included in these studies and the variability of the study parameters (e.g., the type and the number of cells, the number of doses, and at what intervals) preclude determination of the most suitable method for achieving an optimal OA treatment. Given the number of different OA progression scoring methods, it is also difficult to compare the results obtained in these studies [42].

The use of IA injection of MSCs for OA is still in its infancy. The limited quality of some of these studies cautions us about the clinical improvement brought about by the injection of MSCs. Currently, many questions remain unresolved. The stage of OA at which the MSCs should be injected also remains to be determined [50]. The performance of MSCs according to their source (ASC, BM-MSC, and UC-MSC) or type (allogeneic or autologous) has not been compared in any study. Moreover, the optimal number of cells to be injected is not defined. No correlation between cell dose and clinical effect has been proved yet [51]. Better evidence is required through high-quality clinical trials with reproducible methodology. Finally, some potency assays able to assess “a priori” the quality of MSCs are still lacking. Such assays could, however, be very instrumental to precisely address the clinical potential of MSCs before administering as an IA injection.

Limitations of MSC applications

Although there is reason for optimism with regard to the effectiveness of MSCs in the treatment of OA, there is also a need for a convenient cell delivery system. Such a system should be able, first, to transfer and maintain these cells at the lesion site and, second, to ensure viability and functionality of the injected cells. In OA, unlike pathological situations where MSC are systemically injected, MSCs are locally delivered directly into the joint space [52]. Unfortunately, several studies have highlighted several major limitations with regard to the injection of naked MSC suspensions including: (i) massive cell death upon injection because of shear forces upon passage through the needle and potential interactions between the cells and the syringe or needle material [16,53] and (ii) risk of the cells leaking out of the articular space because of the propensity of MSCs to migrate and because of the low level of cell engraftment [15,54].

To overcome this hurdle, cells can be trapped in three-dimensional (3D) hydrogels or scaffolds to enhance their retention at the targeted tissue and to protect them from shear stress damage upon injection. Hydrogels, particularly natural polymers such as collagen or hyaluronic acid, create a biomimetic environment that enhances cell viability and functionality. The stiffness and elasticity of this environment must also be considered, as MSCs preferentially remain in high elastic modulus regions where they accumulate [55]. However, the large size of these devices does not allow for their administration by injection through a needle, instead requiring surgical intervention with its associated adverse effects. Injection requires devices that are preferentially spherical and elastic, with a size that is in keeping with the characteristics of the needle. An attractive alternative is to encapsulate cells to form spherical particles to protect the cells and to promote administration by the injectable route.

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MSC encapsulation

Principle

MSC encapsulation consists of entrapping the cells within a spherical structure, referred to as a particle, which is composed of biocompatible biomaterials.

Such spherical particles are usually classified into two groups depending on their architecture: capsules and spheres (Fig. 1) [56]. Capsules are spherical particles composed of a membrane formed by a continuous semipermeable film of a crosslinked polymer that isolates the encapsulated cells within the core of this structure. They are referred to as a "shell" system. Spheres are solid particles composed of a continuous scaffold of crosslinked polymer in which the encapsulated cells are dispersed. They correspond to a matrix system. The generation of a capsule or a sphere is determined by the nature of the selected biomaterial and the operating conditions to generate them. Hydrophilic biomaterials are usually more appropriate for sphere generation, whereas hydrophobic biomaterials are recommended for capsules [57].

Cell encapsulation is carried out after mixing the cells with polymeric solutions, and these solutions are allowed to crosslink under operating conditions depending on the nature of the polymer. This innovative approach was first proposed in 1980 by Lim and Sun who encapsulated pancreatic cells in alginate before injecting them into diabetic rats to avoid immunosuppressive effects [58]. Since then, numerous studies have focused on the therapeutic potential of cell encapsulation for both regenerative medicine or long-term drug delivery [59].

MSC encapsulation in OA treatment

MSC encapsulation is an approach that could overcome the above-mentioned limitations of the injection of free cells and thus enhance their therapeutic use. The entrapment of cells in particles

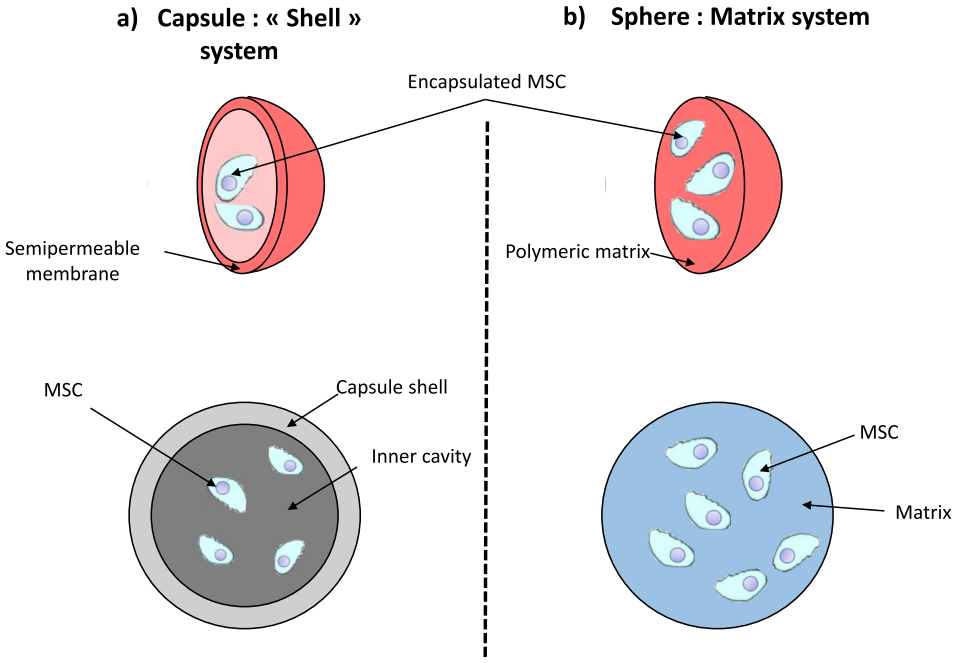

Fig. 1. Spherical particle inner morphology: a) the capsules consist of an inner solid or liquid cavity in which the cells are entrapped, surrounded by a semipermeable polymeric membrane; b) the spheres are composed of a polymeric matrix in which the cells are embedded.

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protects them from the mechanical forces during injection. Indeed, cells are subjected first to shear stresses when aspirated through the syringe needle and second to stretching forces and deformation owing to extensional flow during ejection trough the syringe needle, thereby leading to cell death [53]. The damage inflicted on the cells by injection is related to both the inner diameter and length of the needle. An interesting study has compared the viability of MSCs, encapsulated or not in alginate, after injection through a 28-G needle. The viability of the unencapsulated MSCs was only 58% versus 89% when encapsulated. The presence of the biomaterial, especially shear-thinning hydrogels, around the cells decreases the mechanical forces that they are subject to during injection [16].

MSC migration can also be reduced by better engraftment. Thus, after the injection of labeled and agarose-encapsulated MSCs in an animal model, the cells could still be detected at the injection site 6 months later [18]. By contrast, when directly injected, the MSCs were only detected at the administration site during the first 2 days. More recently, allogeneic MSCs were embedded in cylindrical alginate constructs and implanted subcutaneously in rats [17]. The embedded MSCs could be detected at the implantation site throughout the 5-week study period, whereas MSCs that were suspended in saline and injected subcutaneously were only detectable for 2 weeks. Moreover, the implantation of embedded human xenogeneic MSCs in rats was well tolerated, without any macroscopic signs of inflammation. The alginate construct prevented cell migration, while also ensuring their viability.

To develop a system to tackle the low-grade inflammation described in OA, the drug delivery system must be able to maintain cell viability and functionality for as long as possible. To meet this challenge, the device must comply with several requirements. Briefly, as shown in Fig. 2, diffusion into the polymeric device must be controlled to ensure the diffusion of (i) small molecules such as nutrients or oxygen, (ii) proinflammatory factors to stimulate the MSCs encapsulated in the particle, (iii) waste products, (iv) small molecules such as trophic factors, and (v) microvesicles and exosomes produced by entrapped cells out of the particle. Furthermore, the device must prevent components of the host's immune system (including antibodies and immune cells) from interacting directly with the MSCs to avoid any activation of the immune response, thereby assisting with preservation of the MSCs [59].

Bidirectional diffusion and control of the porosity are essential factors for MSC survival and biofunctionality. The encapsulation into particles should prevent (i) interaction between immune cells and the encapsulated MSCs and (ii) migration of the encapsulated cells beyond the confines of the particles. Most hydrogels used for cell encapsulation have a molecular weight cut-off between 50 kDa and 100 kDa to exclude whole cells, cytotoxic antibodies, and other deleterious complex macromolecules [59]. The diffusion of solutes through the particle matrix obeys Fick's law: solutes diffuse from regions of high concentration to regions of low concentration [60]. This diffusion can be influenced by the solute molecular weight, possible solute–matrix interactions (e.g., electrostatic, physical, or chemical), and solute–solute interactions (e.g., aggregation and self-assembly). Additionally, the diffusion of solutes is affected by properties of the particle such as its elasticity, pore size, pore distribution, and swelling of the particle matrix [60,61].

The mechanical properties and structural stability of the particles are also important parameters for successful MSC encapsulation. The particles must have sufficient mechanical properties to protect the encapsulated cells. Recent studies have underscored the high potential of viscoelastic particles to allow for a faster relaxation time (i.e., the ability of the particles to return to their equilibrium state after a mechanical strain) during and after injection to protect the encapsulated cells [62,63]. Moreover, the structural stability of particles ought to be only minimally affected by joint friction. A loss of particle integrity leads to release of the encapsulated MSCs that could then migrate to other tissues. However, excessively stiff particles can reduce the viability of the encapsulated cells because of oxidative stress and alteration of the diffusion properties of the particles [64].

The particle size is another parameter that needs to be considered. The particle diameter must be suitable for *in vivo* applications. The choice of particle size is governed by a compromise between the nature of the site of injection, the animal model, the targeted number of cells to be injected, and the desired degree of exchange between the particles and their external environment. A small particle size (i.e., <350 μm) allows for better diffusion throughout the capsule than medium (i.e., 350–700 μm) or larger sizes (i.e., >700 μm) for which diffusion to the core of the matrix is slower [59]. A reduced flow to

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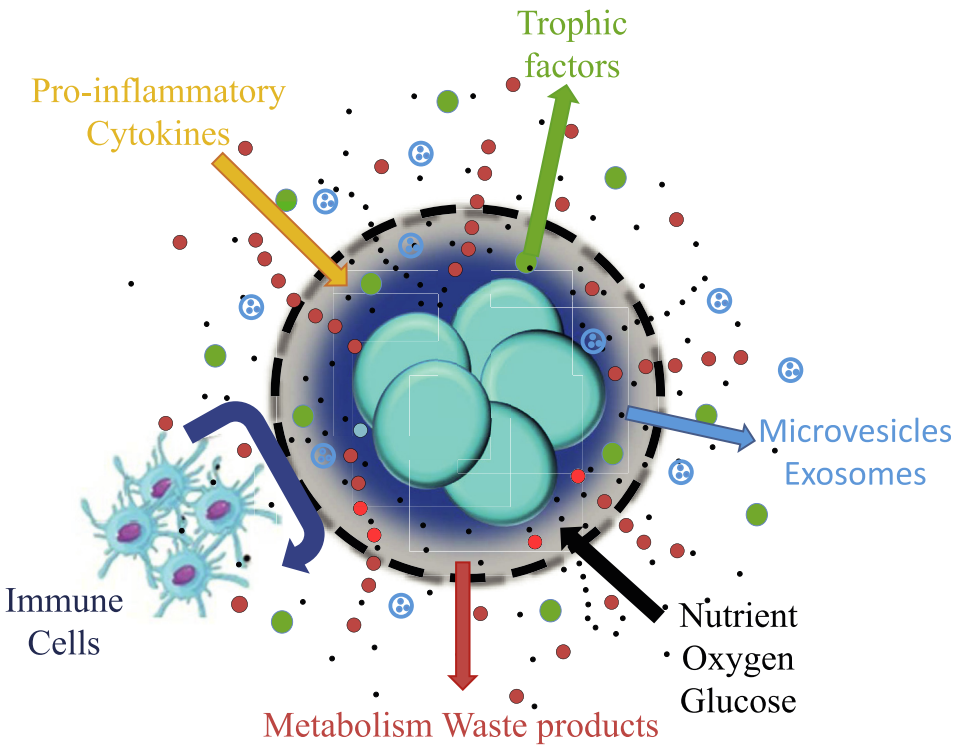

Fig. 2. Schematic illustration of MSC encapsulation. MSC encapsulation is based on the entrapment of cells within a spherical polymeric device. Protection of the inner cells from mechanical stress is ensured, whereas the bidirectional diffusion of nutrients, oxygen, growth factors such as cytokines, microvesicles, and waste products is allowed. Moreover, the semipermeable device prevents components of the host's immune system (including antibodies and immune cells) from interacting directly with the MSCs, thereby avoiding the activation of an immune response.

the inner areas of the microparticle could be detrimental to cell viability [65]. Particle geometry is a parameter that also has an impact on the injectability and biocompatibility of hydrogels. Indeed, it has been reported that particles with a nonspherical shape or acute angles are less suitable for injection (owing to their poor flow rates), and most importantly, they induce inflammation by triggering a substantial degree of foreign body responses *in vivo* [66,67].

Table 2
The main technologies used for cell encapsulation.

Method	Shape uniformity	Size uniformity	Advantages and limitations	MSC encapsulation
Extrusion	Medium	Medium	***Advantages:*** easy, fast, and low cost. ***Drawbacks:*** limited particle uniformity.	[74–77]
Emulsion	High	Medium	***Advantages:*** high encapsulation efficiency and control of therapeutic molecule diffusion. ***Drawbacks:*** limited particle uniformity and shear stress.	[71,78]
Fluidics	High	High	***Advantages:*** high encapsulation efficiency, particle uniformity, and requirement of only a small amount of reagents. ***Drawbacks:*** low production yield.	[79,80]
3D printing	High	High	***Advantages:*** high particle uniformity and encapsulation efficiency. ***Drawbacks:*** not yet sufficiently studied and expensive.	[81,82]
Photolithography	High	High	***Advantages:*** high particle uniformity and encapsulation efficiency. ***Drawbacks:*** not yet sufficiently studied, complexity, and not always adequate for cell encapsulation.	[83]

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Table 3
Biocompatibility evaluation endpoints [87].

Medical device categorization by			Biological effect			
Nature of Body Contact		ContactDuration A – limited(≦ 24 h) B – prolonged(>24 h to 30 d) C – permanent (>30 d)	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Acute Systemic Toxicity
T	Contact					
Surface device	Intact skin	A	X	X	X	
		B	X	X	X	
		C	X	X	X	
	Mucosal membrane	A	X	X	X	
		B	X	X	X	O
		C	X	X	X	O
	Breached or compromised surface	A	X	X	X	O
		B	X	X	X	O
		C	X	X	X	O
External communicating device	Blood path, indirect	A	X	X	X	X
		B	X	X	X	X
		C	X	X	O	X
	Tissue/bone/dentin	A	X	X	X	O
		B	X	X	X	X
		C	X	X	X	X
	Circulating blood	A	X	X	X	X
		B	X	X	X	X
		C	X	X	X	X
Implant device	Tissue/Bone	A	X	X	X	O
		B	X	X	X	X
		C	X	X	X	X
	Blood	A	X	X	X	X
		B	X	X	X	X
		C	X	X	X	X

Polymers for MSC encapsulation and their requirements

Several natural or synthetic polymers have been used to encapsulate MSCs [62]. Each type of polymer has both strengths and weaknesses.

Natural polymers (e.g., alginate, hyaluronic acid, agarose, chitosan, collagen, and fibrin) have been studied the most owing to their biocompatibility and the ease of their polymerization processes. However, to obtain them from living organisms, they require extraction processes involving harsh chemical treatments, as well as extensive purification to ensure the absence of residual toxins that could induce immune responses [68,69]. Moreover, natural polymers have poor mechanical properties and are difficult to modulate, and in some cases, the chemical modifications that were applied increased the toxicity [70]. To optimize *in vivo* applications of encapsulated MSCs, several polymers have been used in combination to form copolymers. This strategy improves the mechanical properties of the resulting hydrogels, thereby making them suitable for cell therapy [71].

Synthetic polymers are generally thought to have better mechanical properties and chemical stability. Modulation of their properties to tailor the generated particles to the requirements of the tissue in question is easier than with natural polymers. Furthermore, there is a higher level of reproducibility of the generated particles [69,72]. However, MSC encapsulation in synthetic polymers has not been described as much as with natural polymers. It appears that for some of these polymers, the cross-linking requires harsh conditions such as nonphysiological pH or temperatures that are inappropriate for cell encapsulation [69]. Polyethylene glycol (PEG), methacrylate, polyacrylamide (PAM), polycaprolactone (PCL), and polyvinyl alcohol (PVA) are examples of synthetic polymers that have undergone evaluation for MSC encapsulation.

The success of encapsulated MSCs for OA treatment relies on their engraftment in joints and their stimulation by different proinflammatory factors present in the environment that can continuously induce

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Biological effect							
Material-Mediated Pyrogenicity	Subacute/ Subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/ Developmental Toxicity	Degradation
O	O		O					
O	X	X	O		O			
O								
O	O		O					
O			O		O	O		
O				X				
O				X				
O	X	X	O	X	O	O		
O								
O	X	X	X					
O	X	X	X		O	O		
O		O		X				
O	X	X	X	X				
O	X	X	X	X	O	O		
O								
O	X	X	X					
O	X	X	X		O	O		
O		O	X	X				
O	X	X	X	X				
O	X	X	X	X	O	O		

X = ISO 10993 recommended endpoints for consideration.
O = Additional FDA recommended endpoints for consideration.

the production of bioactive molecules as long as the cells are alive. This prolonged production and release of anti-inflammatory molecules depends on the stability, biocompatibility, durability, and diffusional properties of the device [73]. For a prolonged release for a period of several months, the selected polymer should not be resorbable for the duration of the desired effect. Agarose and PEG, which are nonbiodegradable polymers, have ample potential for the development of long-term drug delivery by encapsulated cells.

The main techniques for cell encapsulation

Several techniques have been developed for cell encapsulation (Table 2). The most widely used are extrusion, emulsion, and fluidic techniques. 3D printing and photolithography are new approaches to produce well-defined constructs.

The choice of cell encapsulation method depends on the selected polymer, especially its physicochemical characteristics and its crosslinking mechanism.

For efficient encapsulation, the selected technique must generate particles that have a monodisperse size and maintain the viability, multipotency, and biofunctionality of the MSCs. The vast majority of current methods are able to meet these requirements. However, they need to be further improved to (i) reduce encapsulation costs, (ii) accelerate the time of particle generation to reduce "cellular stress" phenomena, (iii) generate particles with reproducible sizes and shapes, and (iv) maximize particle generation and cell encapsulation yields.

Encapsulated MSC applications in cell therapy

Encapsulated MSC applications have been used lesser for cell therapy than for cartilage engineering. We recently demonstrated that, *in vitro*, human MSC encapsulation supports the retention of their

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ability to secrete therapeutic factors [84]. Human ASCs were encapsulated in either alginate or a semisynthetic polymer (silanized hydroxypropyl methylcellulose (Si-HPMC)). Both the alginate and Si-HPMC particles supported cell survival (i.e., greater than or equal to 86% for up to 1 month). When the encapsulated MSCs were stimulated for 72 h with proinflammatory molecules (e.g., TNF-α + IFN-γ at 20 ng/ml), they responded to this stimulation by increasing their secretion of IDO, PGE_2, and HGF. In another study, Leijs et al. entrapped human BM-MSCs in alginate [17]. The cells were stimulated for 24 h with TNF-α + IFN-γ at 50 ng/ml. The encapsulated MSCs responded to the inflammatory stimuli in a manner similar to that of MSCs in a monolayer culture. In addition, the MSC-alginate beads secreted immunomodulatory and trophic factors (e.g., IL-6 and IDO) after 30 days of *in vitro* culture. Moreover, when the encapsulated MSCs were cocultured with activated T cells, the authors observed an inhibition of T cell proliferation and activation for up to 30 days. These studies were performed with large-diameter particles (1–8 mm) that are unsuitable for administration through the injectable route. These experiments only demonstrate the maintenance of MSC immunomodulation after encapsulation. However, the concentrations (20 and 50 ng/ml) of proinflammatory cytokines used to stimulate the encapsulated MSCs do not necessarily reflect the concentrations of these cytokines in the pathophysiology of OA. Proinflammatory cytokine concentrations in OA are significantly less than 20 or 50 ng/ml. Moreover, many questions remain unanswered. These questions relate to (i) the *in vivo* stability of the particles; (ii) the optimal number of MSCs (and therefore, the number and the size limit of the particles) needed to obtain a therapeutic effect, and (iii) the optimal stage of OA to inject encapsulated MSCs. *In vivo* studies would provide an answer to these questions that are of relevance for a long-term implantation.

Safety requirements for MSC encapsulation

Clinical translation still requires several questions to be resolved. One of the major issues is with regard to the safety of encapsulated MSC administration in humans.

Polymers are considered to be pharmaceutical excipients in drug delivery systems [85]. They are not intended to exert therapeutic effects but rather to act to control product delivery (i.e., therapeutic factors secreted by the encapsulated MSCs). Therefore, these polymers not only need to exhibit the characteristics required for their technological function but must also meet a suitable level of quality (such as extended-release formulations) and safety requirements. If encapsulated cells are to be used in humans, the polymeric particles need to be biocompatible. In this context, biocompatibility can be defined as “the ability of a material to locally trigger and guide nonfibrotic wound healing, reconstruction, and tissue integration” [86]. The ISO 10933 standard comprises a series of standards for evaluating the biocompatibility. Table 3 provides a framework for the development of a biocompatibility evaluation. The nature of the tissue in contact with the particles (articular) and the expected duration of *in vivo* persistence (>3 months) require the application of a certain number of evaluations.

Therefore, the biocompatibility evaluation must include cytotoxicity, sensitization, irritation, acute systemic toxicity, subacute/subchronic toxicity, genotoxicity, and implantation endpoints. In addition, the FDA has recommended three other endpoints, namely, material-mediated pyrogenicity, chronic toxicity, and carcinogenicity. These evaluations are based on *in vitro* and *ex vivo* test methods and on animal models. This biocompatibility depends on several important parameters including (i) sterilization conditions and (ii) particle structure, morphology, and stability.

Summary

OA is a complex disease that affects a steadily increasing number of individuals as the population ages. Preclinical studies and clinical trials have highlighted the therapeutic potential of MSCs in OA treatment. However, while it has therapeutic merit, the injection of MSCs has major limitations that include cell death upon injection and pronounced leakage from the injection site. MSC encapsulation overcomes both of these limitations. This strategy hence has a promising future, and published preclinical studies have yielded encouraging results with regard to OA. However, optimization of the most suitable dose of MSCs, biomaterials, and particle manufacturing will be required for successful clinical translation of MSC encapsulation to OA treatment.

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Practice points

- MSCs have generated significant medical interest, as they exert their therapeutic properties by secretion of bioactive trophic factors.
- IA injection of MSCs is safe and induces very few side effects.
- The injection of MSCs has major limitations that may be overcome by cell encapsulation.
- Various polymers and manufacturing process are available for MSC encapsulation.

Research agenda

- Existing polymers and encapsulation manufacturing processes require more research and development to optimize the applications of MSCs after encapsulation.
- The secretion of therapeutic bioactive factors after MSC encapsulation has not yet been investigated adequately to allow their potential application in OA treatment.
- There is a need to determine the optimal number of encapsulated MSCs and the injection timing to obtain a therapeutic effect.

Conflicts of interest

There are no potential conflicts of interest that could inappropriately influence the content of this article.

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